



The coastal medicinally important herb species, *Heliotropium indicum* L. (Boraginaceae): pollination and danaid butterfly association

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General Note

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ABSTRACT

Heliotropium indicum is a coastal herb species which flowers throughout the year with concentrated flowering during wet season. The floral characters such as small light violet plate-shaped flowers with tubular corolla, concealed nectar and aggregate arrangement of flowers on one side of the swinging inflorescence constitute butterfly pollination syndrome. The flowers are exclusively pollinated by danaid adult butterflies while collecting nectar. Further, these butterflies use spent as well as withering inflorescences as alkaloid source for use in the courtship behavior and protection from their predators. Fruit set is substantially high indicating the function of facultative autogamy but seed set rate is very low which is attributed to genetic ability, availability of soil

nutrients and extraction of alkaloids by danaid butterflies from spent as well as withering and withered inflorescences. Low seed set rate is compensated by year-long flowering and fruiting.

Key words:

Heliotropium indicum, butterfly-pollination, danaid butterflies, alkaloids.

1. INTRODUCTION

The family Boraginaceae comprises about 135 genera and 2,600 species distributed mostly in tropical, subtropical and temperate regions of the world (Retief and Van Wyk 2008). It is characterized by a scorpioid cymose inflorescence, a gynobasic style, and a two-part ovary that breaks into four nutlets (Buys and Hilger 2003). In Boraginaceae, pyrrolizidine alkaloids are quite common constituents across different genera.

Heliotropium is a large and complex genus with about 270-300 species distributed in temperate and tropical regions of the world (Forther 1998; Gurib-Fakim 2008). Its name is derived from two Greek words "Helios" meaning "sun" and "tropein" meaning "to turn". Together, the word *Heliotropium* refers to the inflorescence with its rows of flowers turning to the sun (Chittenden 1951; Ghosh et al. 2018). The genus characteristics include the presence of terminal or axillary scorpioid cyme with small flowers, 5-partite lanceolate calyx lobes, 5-lobed tubular corolla, 5 free stamens attached to the corolla tube, incompletely bi-locular ovary (Ali and Nasir 1989). *H. indicum* is highly endemic in India and Bangladesh while it is found in different parts of the world, especially in many African countries (Ghosh et al. 2018). In a review on traditional uses of *H. indicum* by Ghosh et al. (2018), it is stated that this plant is used to treat various diseases throughout the distribution range of the species including India.

Bull et al. (1968) reported that Danaid butterflies, especially males are attracted to certain plants, mainly belonging to *Senecio*, *Eupatorium* (Asteraceae), *Heliotropium*, *Tournefortia* (Boraginaceae) and *Crotalaria* (Fabaceae) which are well known sources of pyrrolizidine alkaloids. These butterflies suck at withered leaves, stems and seed pods for alkaloids (Pliske 1975a,b). *H. indicum* leaves contain alkaloids such as trachelanthamidine and pyrrolizidine alkaloid precursor amines, putrescine, spermidine and spermine (Ghosh et al. 2018). Its flowers and damaged stems are the sources of pyrrolizidine alkaloids and these are sequestered by most ithomiine adult butterflies, usually males (Brown 1984). Further, its dead stems are also the sources of pyrrolizidine alkaloids which have role in the defense and reproduction of butterflies which sequester them (Brown 1984; Drummond 1984).

In India, two herb species of *Heliotropium*, *H. curassavicum* and *H. indicum* have been reported to distributed in coastal wetlands, the former either as a constituent of Indian Mangrove flora (Sudhakar Reddy 2008) and as a mangrove associate in Bhitarkanika National Park, Odisha (Panda et al. 2017) while the latter as a constituent of coastal ecosystems occurring in sandy beaches of western Maharashtra (Gokhale et al. 2011) and as a member species of wetland ecosystems of Srikakulam District, Andhra Pradesh (Mathew et al. 2012). The present study found that *H. indicum* is distributed in coastal areas, occurring both in estuarine zones as well as in areas adjoining coastal belt in Visakhapatnam region, Andhra Pradesh. In this paper, the floral biology of *H. indicum* and the association between this plant and danaid migratory butterflies is described and discussed.

2. MATERIALS AND METHODS

Heliotropium indicum growing as patchy populations in the peripheries of Coringa Mangrove Forest (16°30'–17°00'N and 82°10'–80°23'E) in the deltaic region of East Godavari District, Andhra Pradesh, India were used for the study. Field studies in this Mangrove forest carried out over a period of thirty years by the first author along with different batches of doctoral students revealed that *H. indicum* flowers throughout the year with concentrated flowering during July-November and accordingly the study was conducted during July-November 2019. Field observations on the position of the inflorescence and arrangement of flowers on the inflorescence were made since these features are important for foragers to use them as forage source. Ten mature buds were tagged and followed them until fell off to record the duration of flower life. The same buds were also used to record anthesis schedule and mode of anther dehiscence. The floral morphological and structural details were observed under microscope and recorded to characterize their role in attracting and effecting pollination by foragers. The stigma receptivity was observed by H₂O₂ test as given in Dafni et al. (2005). Fruit set and seed set rates in open-pollinations at inflorescence level were recorded by tagging ten inflorescences prior to their first anthesis and following them until fruit and seed formation. Further, fruit maturation period, fruit dehiscence and seed dispersal were also observed in the field.

The insects visiting the flowers were butterflies only. These butterflies were observed carefully day long for four days in different weeks during flowering season. The hourly foraging visits of each butterfly species were recorded on fifty selected inflorescences.

The data collected was used to calculate the percentage of foraging visits made by each butterfly species per day and to evaluate their relative importance in effecting pollination. Simultaneously, they were observed for their foraging behavior such as mode of approach, landing, probing behaviour, the type of forage they collected, contact with essential organs to result in pollination. Further, field observations were made on alkaloid collection activity by butterflies on spent and withered inflorescences.

3. RESULTS

Heliotropium indicum grows in sunny, water saturated localities close to the landward side mangrove forest (Figure 1a). It also occurs in semi-dry localities along the coastal belt. It is an annual, branched herb with slightly woody base. The stem is erect and hairy with distinctively stalked ovate to oblong-ovate leaves. The plant thrives well with robust growth and intense flowering in wet areas while it shows scanty growth with moderate flowering in semi-dry localities. The flowering occurs throughout the year but intense flowering occurs only during July-October; it is very prolific and quite prominent in wet areas while its flowering intensity is dependent on the moisture status of the soil in semi-dry localities. The flowers are aggregated and develop apically on only one side of the cyme inflorescence which is terminal, up to 22 cm long and extends from the end of the branch; the tip of the inflorescence is prominently coiled. Individual inflorescences produce 22 ± 78 flowers which are open sequentially from the base to the tip (Figure 1b).

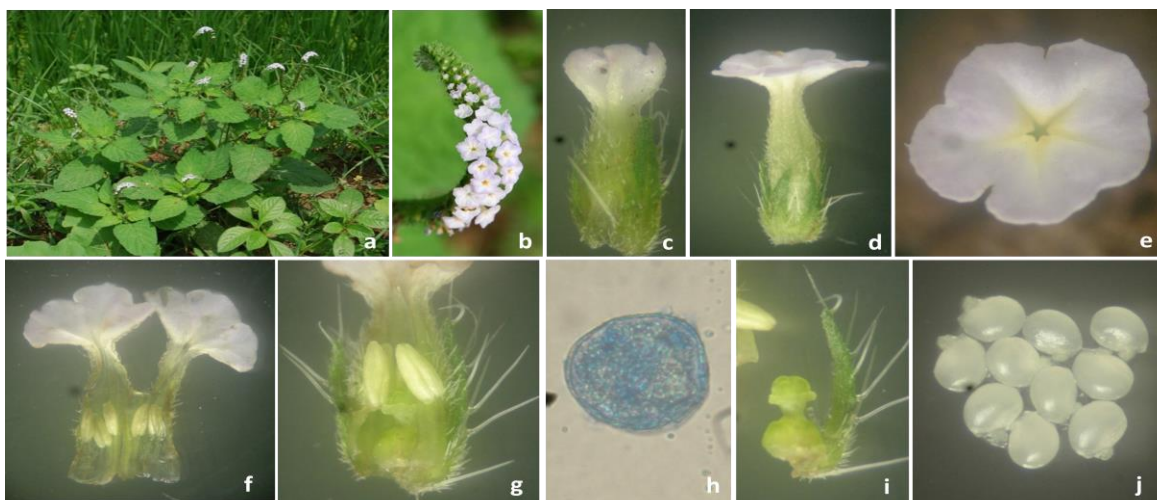


Figure 1. *Heliotropium indicum*: a. Habit, b. Inflorescence in flowering phase, c. Mature bud, d. & e. Pale violet flowers, f. & g. Position of stamens and stigma in the flower, h. Pollen grain, i. Ovary, style and stigma, j. Ovules

The flowers are small, 1.5 mm long, 5 mm in diameter, pale violet with yellow throat (Figure 1d,e), actinomorphic and bisexual. Calyx consists of 5 green linear to lanceolate-shaped sepals fringed with hairs. The corolla tube is 4-5 mm long with five dentate rounded fused petals forming a plate shape. The stamens are 5, free, 3 mm long, included and attached to the corolla tube; the anthers are sub-sessile, elongated and light yellow colour. Ovary is 4-lobed, 4-ovuled with a terminal erect style with conical stigma (Figure 1i,j) which is situated just below the anthers during and after anthesis (Figure 1f,g). Fruits mature within a month and the calyx being persistent harbours the growing fruit together with its seeds. In open-pollinations, fruit set rate is 93% and seed set rate 25%. Fruits are dry, indehiscent and 3.5 mm long with or without united ovoid, ribbed nutlets, separating into 2 nutlets and each nutlet is 2-celled and beaked. After maturation, fruits detach and fall off within the vicinity of mother plant. Ground-fallen fruits gradually decompose and expose seeds for germination and establishment subsequently.

The mature buds (Figure 1c) open during 0700-1600 h with more anthesis at 1030-1200 h. Anther dehiscence takes place during anthesis by longitudinal slits. The pollen grains are light yellow, semi-dry, prolate, spheroidal and $18.26 \pm 3.49 \mu\text{m}$ in size with psilate exine sculpturing (Figure 1h). The stigma attains receptivity 30 min after anther dehiscence and remains so in wet stage until 1800 h of the day of anthesis. Individual flowers secrete traces of nectar and situated at the base of corolla. The corolla remains in place in withered state even after fruit development in many fruited flowers.



Figure 2. *Heliotropium indicum*: a-h. Nymphalid butterflies – a-d. Nectar foraging activity on flowers– a. *Tirumala limniace*, b. *Danaus chrysippus*, c. *Danaus genutia*, d. *Euploea core*, e-h. Sap-cum-alkaloid collection activity on spent inflorescences – e. *Tirumala limniace*, f. & g. *Danaus chrysippus*, h. *Euploea core*.

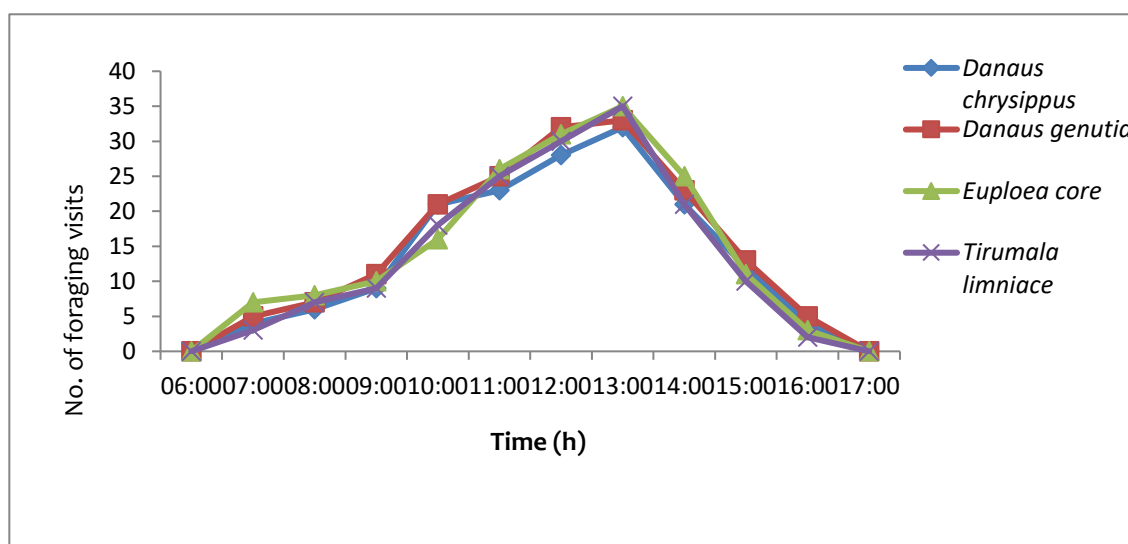


Figure 3. Hourly foraging activity of danaid butterflies on the flowers of *Heliotropium indicum*

The flowers were consistently foraged during day time from 0700-1600 h with maximum foraging activity during 1000-1400 h by four species of butterflies, namely, *Danaus chrysippus* L. (Figure 2b), *D. genutia* Cramer (Figure 2c), *Euploea core* Cramer (Figure 2d) and *Tirumala limniaceae* Cramer (Figure 2a) (Family: Nymphalidae, sub-family: Danainae) (Figure 3). Of these, *D. genutia* and *E. core* each made 26% and *D. chrysippus* and *T. limniaceae* each made 24% of total foraging visits made in a day. Individuals of these butterflies visited the flowering inflorescences singly and also different species never visited the same inflorescence at the same time. They approached the inflorescences in upright position and landed either at the base or tip of the inflorescence prior to probing the flowers for nectar. The terminally situated inflorescences above the foliage with light violet flat-topped or plate-shaped aggregated flowers are very conspicuous to facilitate these butterflies to identify the forage source even from a long distance. After landing, the butterflies inserted their proboscides into the corolla throat and through the stamens and stigma situated inside the corolla to access nectar and in the process, the proboscis strikes the dehiscent anthers and transfers the pollen onto the stigma effecting pollination. The proboscis of the butterflies brushed with the pollen was visible to the naked eye. In subsequent flower visits by these butterflies to the inflorescences of the same or different plants was considered to be effecting geitonogamous and xenogamic pollination. The aggregate arrangement of the flowers on the inflorescence was found to be enabling the visiting butterflies to collect nectar from most of the flowers in a single visit by minimizing search and flight time for the flowers. Further, the butterflies visiting the flowers for nectar were very slow in movement from inflorescence to inflorescence on the same or different

conspecific plants and that slow movement was considered to be a "sluggish behavior" exhibited by them as a result of intoxication by alkaloids present in the nectar.

All four species danaid butterflies also consistently collected sap consisting of pyrrolizidine alkaloids from the just spent, withering and withered inflorescences throughout the day; 3-8 individuals of each butterfly species aggregated and visited the spent inflorescence at the same time harmoniously for the collection of alkaloids (Figure 2e-h). Individuals of different danaid butterflies never aggregated at the same time and at the same inflorescence for the collection of alkaloids. These butterflies never made any attempts to suck alkaloids from the fresh or damaged leaves or stem.

4. DISCUSSION

Heliotropium indicum is a typical herb and thrives well in different habitats of coastal areas. It is highly prolific with robust growth in wet areas and scanty with weak growth in semi-dry areas. In wet areas, the plant appears throughout the year and displays flowering and fruiting continuously at population level in wet areas, however, intense flowering and fruiting occurs during July-November. In semi-dry areas, the plant shows vegetative growth, flowering and fruiting sequentially during July-October and disappears thereafter. The differential functions of vegetative growth, flowering and fruiting in this plant in wet and semi-dry areas are indicative of the effect of water and moisture availability in the soils of these habitats.

In *H. indicum*, the anthesis schedule during bright sunlight hours of the day indicates that the plant is adapted for pollination by day-active foraging insects. The floral characters such as small light violet flowers with narrow tubular corolla and nectar concealed at the corolla base strongly select the foragers that require a more elaborate flower handling and probing behavior to access nectar. Such specialized foragers contact the anthers and stigma using their proboscis while accessing the deeply seated nectar and in the process contribute to self or cross-pollination. The flowers with these characteristics conform to butterfly or psychophilous pollination syndrome (Burkhardt 1964; Faegri and van der Pijl 1979). The present study indicates that *H. indicum* is exclusively pollinated by butterflies, particularly a special danaid group of nymphalidae family butterflies. The flat-topped or plate-shaped flowers arranged in aggregated manner in a linear row on one side of the swinging inflorescence with coiled tip extended above the foliage provide multiple advantages: attraction to butterflies situated far away from the plant, provide comfortable landing and reduce search and flight time, and are also energetically rewarding for butterflies (Neff et al. 1977). The danaid butterflies begin their nectar foraging activity as soon as the flowers of *H. indicum* are open and continue nectar collection activity until the evening. The long duration of anthesis facilitate these butterflies to collect nectar almost day-long and exhibit maximum nectar collection activity during the peak anthesis period. The butterflies by inserting their proboscis through corolla throat and then through anthers and stigma effect self- or cross-pollination. Since the flowers are weakly protandrous, the opportunities for self-pollination are very high and this statement is substantiated by high rates of fruit set. However, seed set rate is substantially low when compared to fruit set rate and this situation is relatable to the extraction of alkaloids by butterflies from spent inflorescences, lack of genetic ability to enhance seed set rate and to the availability of nutrients in the soil environment. The year-long flowering and fruiting strategy displayed by this plant is advantageous to compensate the low seed set rate and propagate sexually as and when seeds are exposed for germination from the indehiscent decomposed fruit on the ground.

Nicolson (2007) stated that pyrrolizidine alkaloids in nectar are inhibitory to generalist-feeding butterflies but attractive to specialist feeders. Some plant species produce mildly toxic or narcotic levels of chemical constituents in their nectars. After imbibing these narcotic substances, pollinator insects become naturally intoxicated, and exhibit more "sluggish" behaviour which increases the time spent within the flower and the chances of successful pollination. Klinkhamer and De Jong (1993) felt that this intoxication of pollinators could lead to undesirable levels of geitonogamous self-pollination. Churi and Sarkar (2020) reported that *Heliotropium indicum* is a nectar plant for *Euploea core*, *Danaus genutia*, *Danaus chrysippus*, *Tirumala limniace* and *Parantica aglea*. Brown (1984) reported that *Heliotropium indicum* leaves, flowers and damaged stems are the source of pyrrolizidine alkaloids for ithomiine adult butterflies, usually males. In the present study, it is found that *H. indicum* flowers are used as nectar source only by danaid butterflies and moths never visited the flowers of this species for nectar. The slow movements of danaid butterflies between inflorescences of the same or different conspecific plants indicate that the nectar could be intoxicating them to exhibit sluggish behavior and increase vector-mediated autogamy and geitonogamy.

Pliske (1975a,b) reported that certain plants containing pyrrolizidine alkaloids attract numerous lepidopteran species belonging especially to Danainae, Ithomiinae, Ctenuchidae, Arctiidae). These lepidopterans congregate on dead shoots and inflorescences of such plants to feed for alkaloids. In many species, visiting lepidopterans constitute nearly all males. In *Heliotropium indicum*, different live parts of the plant are attractive to these Lepidoptera and establish an unusual relationship. The present study shows that the butterflies feed at just spent inflorescences, withering and withered inflorescences of *H. indicum* for pyrrolizidine alkaloids. The uptake of alkaloids does not provide energy, the males require these secondary plant substances as precursors for the

biosynthesis of a pheromone component. In addition, both sexes store them (Edgar et al. 1976; 1979), apparently for defense (Rothschild et al. 1979, Conner et al. 1981). Thus, the alkaloids facilitate mating and deter their predators (Bell 2001). Further, Brown (1985; 1987) reported that these butterflies contribute alkaloids to females via the male spermatophore. These phytochemical alkaloids remain unconverted in the bodies of male as well as female butterflies (Edgar et al. 1976). The alkaloids are bitter in taste and extremely noxious for vertebrates (Mattocks 1973). Therefore, these alkaloids not only serve as pheromone precursors but also as protective substances (Edgar et al. 1976).

Seaman et al. (1989) and Stegelmeier et al. (1999) reported that the members of Boraginaceae *Echium* and *Heliotropium* intermittently poisoned Australian livestock. Peterson et al. (1992) reported that *Heliotropium europaeum* poisoned considerable numbers of livestock, particularly sheep in Australia. In the study area, locals have never reported such food poisoning in the livestock which feed on wild plants including *H. indicum*.

The present study indicates that danaid butterflies display a specialized association with *H. indicum* and use as nectar as well as alkaloid source. These butterflies use alkaloids for the production of pheromone precursors, facilitation of courtship behavior and protection from their predators. The plant lures these butterflies to its nectar and alkaloids in order to be pollinated by them. Therefore, a mutual relationship exists between *H. indicum* and danaid adult butterflies.

5. CONCLUSION

Heliotropium indicum is a coastal herbaceous species. It is valued for its medicinal properties. It is a year-long bloomer with concentrated flowering during wet season. The floral characteristics display butterfly-pollination syndrome and butterflies exclusively of danaid group pollinate the flowers. These butterflies feed on nectar for sugars, other chemical constituents, especially alkaloids and spent and withering inflorescences exclusively for alkaloids. Therefore, *H. indicum* and danaid butterflies display a mutualistic association in which both partners benefit from each other.

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The authors declare no conflict of interest.

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